

Principal Investigator: Kimiko Murakami-Murofushi

Grant Title: Mechanism of the Heat Stress Response *via* the Bioactive Glycolipid, Cholesteryl Glucoside

### **Abstract**

Cholesteryl glucoside ( $\beta$ -CG), a monoglucosylated derivative of cholesterol, is involved in the regulation of heat shock responses.  $\beta$ -CG, which is rapidly induced in response to heat shock, activates heat shock transcription factor 1 (HSF1) leading to the expression of heat shock protein 70 (HSP70) in human fibroblasts. Identification and biochemical characterization of the enzyme responsible for  $\beta$ -CG formation is important for complete understanding of the molecular mechanisms leading to HSP70-induction following heat shock. Recently, we demonstrated that  $\beta$ -CG synthesis was not dependent on UDP-Glucose but glucosylceramide (GlcCer) in animal tissue and human fibroblasts. Thus, we examined the possibility of glucocerebrosidase, a GlcCer-degrading glycosidase, acting as  $\beta$ -CG-synthesizing enzyme. Interestingly, the overexpression of  $\beta$ -glucosidase 1 (GBA1, lysosomal acid  $\beta$ -glucocerebrosidase) led to an increase in cholesterol glucosylation activity in human fibroblasts.



In the present study, we examined whether GBA1 acting as  $\beta$ -CG-synthesizing enzyme or not. Using a cell line generated from Gaucher disease patients with severe defects in GBA1 activity, we found that cholesterol glucosylation activity was very low in the cells. In addition, Cerezyme, a recombinant human GBA1 used in enzyme replacement therapy in Gaucher disease, exhibited Conduritol B-epoxide-sensitive cholesterol glucosylation activity. The optimum pH and temperature for cholesterol glucosylation by GBA1 were at about 5.3 and 43 °C, respectively. Short chain C8:0-GlcCer was the most effective donor for cholesterol glucosylation activity among GlcCer containing saturated fatty acid (C8:0 to C18:0) tested. GlcCer containing mono-unsaturated fatty acid was more preferred substrate for cholesterol glucosylation when compared with GlcCer containing same chain length of saturated fatty acid. These results demonstrate, for the first time, a novel function of GBA1 as a  $\beta$ -CG-synthesizing enzyme. Therefore, our results also reveal a new pathway for glycolipid metabolism in mammals.

### **Reference**

Akiyama H., Kobayashi S., Hirabayashi Y. and Murakami-Murofushi K., Cholesterol glucosylation is catalyzed by transglucosylation reaction of  $\beta$ -glucosidase 1., *Biochem. Biophys. Res. Commun.*, 2013, 441:838-843.